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Effects of the root-feeding nematode *Tylenchorhynchus claytoni* on growth and leaf gas exchange of *Bouteloua gracilis* (Gramineae)

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With 2 Figures

1. Introduction

Herbivores may consume 50% or more of annual aboveground net primary production in grasslands (DETLING, 1988). These effects can be observed by comparing plants in grazed and ungrazed areas. Belowground herbivores, particularly nematodes, may consume equal, or even greater, quantities of biomass which can also affect grassland production (COLEMAN et al., 1976; SMOLIK, 1977; SCOTT, 1979; STANTON et al., 1981; SMOLIK & LEWIS, 1982; INGHAM & DETLING, 1990). SMOLIK (1974) estimated that annual plant consumption by root-feeding nematodes ($39 \, g \cdot m^{-2}$) was nearly twice that of cattle ($22 \, g \cdot m^{-2}$), and INGHAM & DETLING (1984) calculated that nematodes consumed 5 to 20% of the annual net root production of grasses in a North American mixed-grass prairie. This loss is particularly significant considering that half or more of the total primary production in grasslands is allocated to roots (SIMS & SINGH, 1978).

Few studies have examined the physiological responses of plants to belowground grazing. Understanding these responses may help explain the mechanisms by which root loss or damage alter plant production. In North American grasslands, the majority of plant-feeding nematodes are ectoparasites (SMOLIK & LEWIS, 1982; SMOLIK & DODD, 1983) which feed on epidermal cells, root hairs and root tips (KRUSBERG, 1959; RHOADES & LINFORD, 1961; KLINKENBERG, 1963). In this study, we examined the effects of the ectoparasite *Tylenchorhynchus claytoni* STEINER on growth, tillering, photosynthesis, transpiration, and biomass allocation in *Bouteloua gracilis* (H.B.K.) GRIFFITHS (blue grama), the dominant grass of the North American shortgrass prairie.

2. Materials and methods

Seeds of *B. gracilis* were germinated in vermiculite and, 15 days later, seedlings were selected for uniformity and planted separate into 60 pots 12.7 cm diameter. Each pot contained 1000 g Ascalon sandy-loam soil (76% sand, 10% silt, 14% clay) which had been autoclaved to kill endemic nematodes and reinfested with a soil extract (COLEMAN et al., 1984) which included bacteria, fungi and Protozoa from the original soil. The soil was collected at the Long Term Ecological Research site on the USDA-ARS Central Plains Experimental Range in north-central Colorado (STANTON et al., 1981). Twenty days after transplanting, half (30) of the pots were infested with a mean of 766 (SE = 75) *T. claytoni* recovered from alfalfa callus tissue cultures (KRUSEBERG, 1961). At this time there was an average of three tillers per plant. Plants were maintained in a growth chamber with 14-h photo periods, 30/15 °C day/night temperatures, and a quantum flux density (PAR) of $700 \, \mu E \cdot \mu^{-2} \cdot s^{-1}$ at plant height.

One, two, and four months later, 10 randomly selected plants of each treatment were destructively harvested. Tillers were counted and each plant was separated into roots, crowns, leaf sheaths, leaf blades, and flowers and culms. Each was oven-dried at 60 °C for 48 hr and weighed. Nematode population estimates were made from each pot by extracting 100 g of nonrhizosphere soil and all rhizosphere soil with a wet sieving technique followed by modified Baermann funnel extraction (INGHAM & DETLING, 1984). Population densities reported are corrected for extraction efficiency. Root consumption by nematodes was calculated as described in INGHAM & DETLING (1986). Root production was determined by the change in root standing crop during a time interval plus the amount of material removed by consumption during that interval. At 3 months following addition of nematodes, tillers were counted and shoots only were harvested by clipping at 4 cm. These defoliated plants were permitted to regrow and were harvested in month four. During the week preceding each harvest, rates of net photosynthesis (P_n) and transpiration were determined by monitoring CO_2 and water vapor exchange on single leaves of each plant to be harvested, following methods of WILLIAMS & KEMP (1978). For each P_n measurement, the uppermost fully expanded leaf was placed in a plexiglass cuvette (10-cm diameter) equipped with a stirring bar to minimize boundary layer resistance. Temperature was 30 ± 0.5 C, and quantum flux density was 1600 µE · µ^{-2} · s^{-1} within the cuvette. Data were analyzed by ANOVA and Tukey's honestly significant difference (HSD) mean separation test (KIRK, 1968).

3. Results and discussion

Tylenchorhynchus claytoni populations increased slowly throughout the study but the ratio of nematodes per gram of root biomass remained relatively constant (Table 1). These numbers were within the range for total Tylenchida (700 – 8000 · g^{-1} root biomass, X ≈ 2000) observed over the growing season in a northern mixed-grass prairie by INGHAM & DETLING (1984), and similar to values (≈ 1000 · g^{-1} root) calculated from data of STANTON et al., (1981) for a *B. gracilis*-dominated North American shortgrass prairie. The proportion of root biomass consumed by the nematodes increased considerably during the course of the experiment, but only exceeded 1% of root production during one sampling interval (table 1).

Table 1. Population (mean and standard error) and consumption estimates during each sampling interval for *Tylenchorhynchus claytoni* populations in pots containing *Bouteloua gracilis* (N = 10)

Day since planting	Number · pot^{-1}	Number · g^{-1} root	Consumption (mg · pot^{-1})	Percent of root production consumed
35	766 (75)	—	—	—
65	1117 (281)	1498 (399)	3.2	0.4
97	5101 (1703)	2019 (732)	11.2	0.6
167	11258 (1916)	1762 (375)	64.6	1.3
Total	—	—	**79.0**	**1.0**

No difference was found between controls and nematode treatments for biomass allocation patterns (data not shown), tiller production (fig. 1A), shoot growth (fig. 1B), root growth (fig. 1C), photosynthetic rate (fig. 2A), or transpiration (fig. 2B). Consistent with our results (fig. 1), STANTON et al. (1981) found no significant change in shoot biomass of *B. gracilis* when nematodes were reduced with the nematicide carbofuran in the field. By contrast, STANTON (1983) observed a 12% decrease in shoot biomass of potted plants, and INGHAM & COLEMAN (1983) observed a 17% decrease in shoot biomass of *B. gracilis* plants growing in microcosms containing *T. claytoni*. INGHAM & COLEMAN (1983) noted that plants fed upon by nematodes consistently had less root biomass whereas STANTON (1983) observed increases in root biomass at low nematode (*Helicotylenchus exallus* SHER) densities and
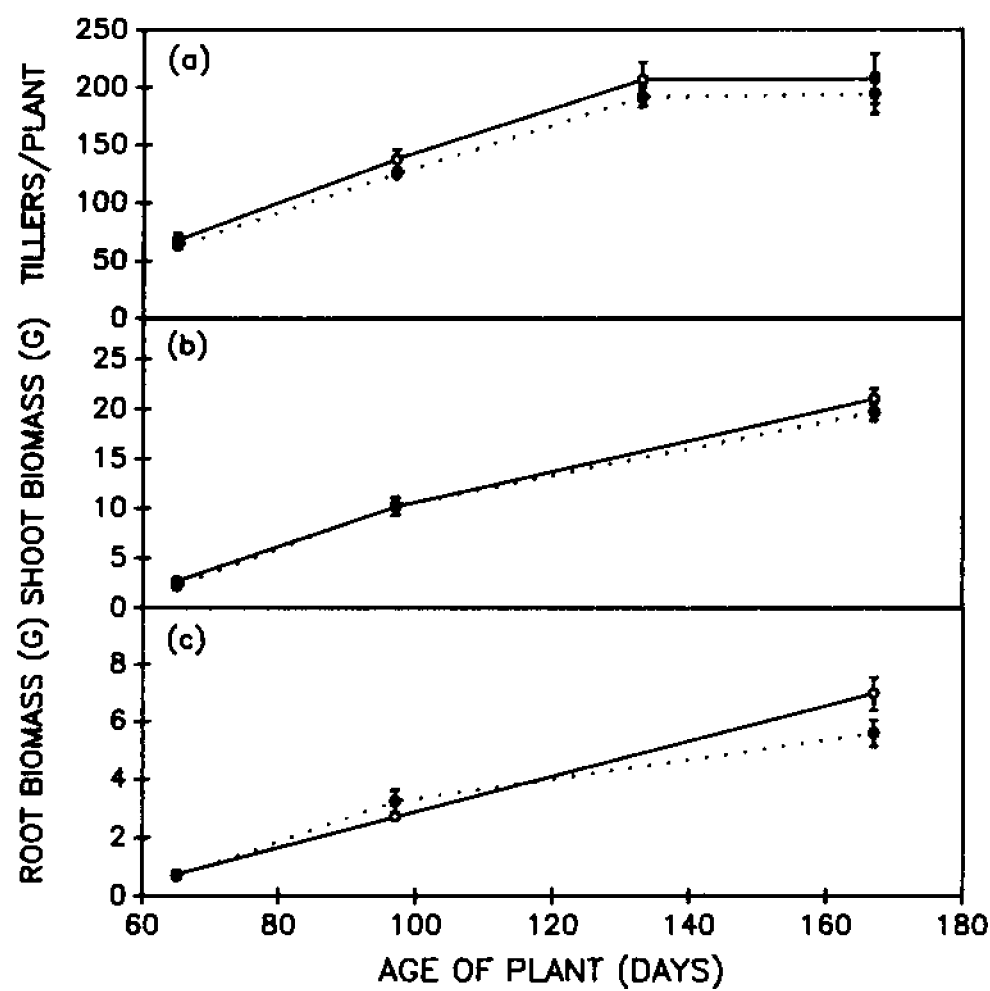
Fig. 1. Tiller production/plant (a), shoot biomass (b), and root biomass (c) for control plants (· ·)
and plants inoculated with *Tylenchorhynchus claytoni* (———). Vertical bars are ± 1 standard error.

decreases at high densities. Root biomass increased $130-150 \text{ g} \cdot \text{m}^{-2}$ when nematode populations in *B. gracilis* field plots were reduced with carbofuran (STANTON *et al.*, 1981) which has no direct effect on root or shoot growth of this species in the absence of nematodes (INGHAM & DETLING, 1982). In general, the growth responses of grasses to root-feeding nematodes tends to be variable. In a review of 30 separate pot studies, INGHAM (1981) found that 27% of the studies reported increased shoot production and 21% reported increased root production with nematodes present. Nematodes decreased shoot or root production in 57 and 68% of the studies, respectively.
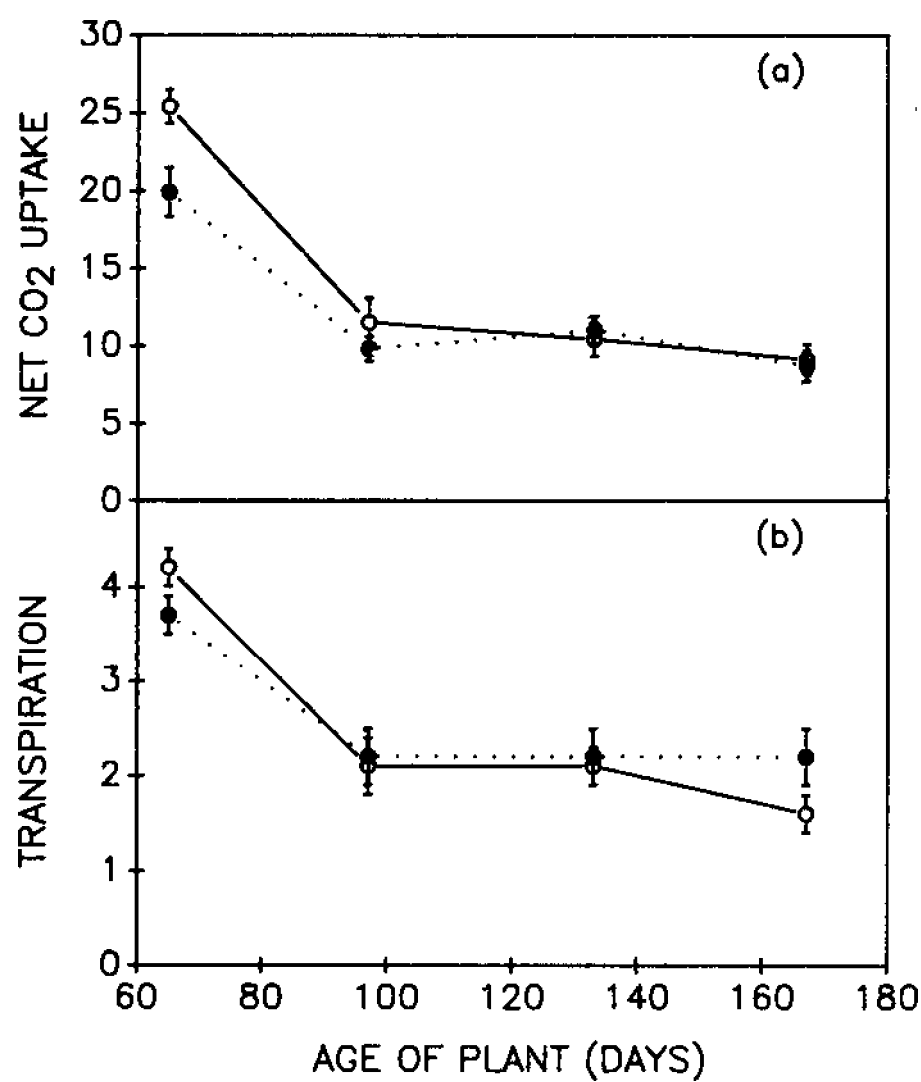

Fig. 2. Net CO_2 uptake rate (μmole $CO_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (a), and transpiration rate (mmole $H_2O \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (b) for control plants (· ·) and plants inoculated with *Tylenchorhynchus claytoni* (———). Vertical bars are ± 1 standard error.

Earlier root-pruning experiments on *B. gracilis* to simulate belowground grazing by root-chewing insect larvae suggested that such damage affected productivity through alterations in net photosynthesis rate, rate of tiller production and carbon allocation patterns (DETLING *et al.*, 1980). However, the potential effects of phytophagous nematodes on these parameters, especially in native plants, have been virtually ignored. INGHAM & DETLING (1986) found that while defoliation stimulated photosynthesis and transpiration, root-feeding by ectoparasitic nematodes had no statistically significant effect. Since plant production of *B. gracilis* was not significantly affected (fig. 1) by the observed population densities of *T. claytoni* in the current study (table 1), it is not surprising that we did not measure detectable differences in any of these three parameters (fig. 2). Greater levels of root consumption such as those recorded for *T. claytoni* on *Bouteloua curtipendula* (MICHAUX) TORREY [INGHAM & DETLING, 1986] or higher numbers per g root biomass as observed by INGHAM & COLEMAN (1983) may have had measurable effects on production and physiology.

Although *T. claytoni* levels (per g root biomass) in these laboratory experiments (table 1) were similar to Tylenchida population densities observed in the field (STANTON *et al.*, 1981; INGHAM & DETLING, 1984), caution should be exercised when attempting to extrapolate from our results to the field. Our observations indicated that the live root/dead root biomass ratio is substantially greater in the laboratory-grown plants. Thus, the number of nematodes per unit of live root biomass may be significantly higher in the field. In addition, effects of other phytophagous nematodes in grasslands may be greater than those of *T. claytoni*. Finally, our laboratory experiments were conducted on well-watered plants at near optimal growth temperatures. Plant growth and physiological responses to nematodes may be different under periodic environmental stresses which are frequently observed in temperate North American grasslands. In addition, there are often greater population densities of root-feeding nematodes (per g root biomass), and thus greater feeding intensity, on grasses which are stressed than on unstressed or less stressed plants. These stresses may include low water and low nutrients (SMOLIK & MALEK, 1973; SMOLIK & DODD, 1983), grazing (INGHAM & DETLING, 1984) or low light and low nutrients (INGHAM & COLEMAN, 1983). Similarly, SMOLIK (1982) observed that *Tylenchorhynchus robustoides* THORNE et MALEK reached higher densities and reduced clipping weights by a greater percentage when the cool season grass *Agropyron smithii* Rydb. was stressed by above optimum temperatures (30 °C) than when the warm season grass *Buchloe dactyloides* (NUTT.) ENGELM. was grown at the same temperatures.

Some nematode species such as root-knot nematodes (*Meloidogyne naasi* FRANKLIN; SIKORA *et al.*, 1972) and sting nematodes (*Belonolaimus longicaudatus* RAU; BOYD *et al.*, 1972) can be highly pathogenic to certain grasses. However, in a shortgrass prairie dominated by *B. gracilis*, the majority of plant-parasitic nematodes were stunt nematodes (*Tylenchorhynchus spp.* and related genera), pin nematodes *(Paratylenchus spp.)* and Tylenchidae *(Tylenchus spp.* and related genera) (SMOLIK & DODD 1983) which, like *T. claytoni*, are much less pathogenic to grasses (COURSEN & JENKINS, 1958; BRIIDGE & HAGUE, 1974). The effects of these nematodes on primary production may be from root-consumption by high nematode densities which may be greater than 1 million/m^2 (SMOLIK & DODD, 1983). The total impact on system productivity, however, will be a function of the population densities of all plant-parasitic species, their host-parasite relationships and the influence of other environmental stresses. Clearly, these all represent areas in which research is needed before an accurate conceptual model of the ecological relationship between native grassland plants and phytophagous nematodes can be formulated.

3. Acknowledgments

6. References

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Shoot, root and tiller production, net photosynthetic rates, transpiration rates, and biomass allocation patterns of *Bouteloua gracilis* (blue grama) were compared among controls and plants inoculated with the ectoparastic nematode *Tylenchorhynchus claytoni*. Total nematode populations increased slowly but number per gram of root biomass remained relatively constant since root biomass increased as well. Nematode densities (1,500−2,000 per g root) were similar to those observed in native North American grasslands. However, calculated nematode consumption exceeded 1% of root production during only one sampling period. No significant differences were observed between control and inoculated plants for any of the measured parameters. These results suggest that *T. claytoni* has less effect on primary production-related processes than might be assumed from estimates of annual consumption by root-feeding nematodes in grasslands. However, the total impact on system productivity will be a function of the density of all plant-parasitic nematodes, their host-parasitic relationships and other environmental stresses.
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